University of California/Davis Interdepartmental Conference on Gram-Negative Septicemia

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Gram-negative septicemia remains one of the most serious forms of hospital-acquired infection. The most consistently virulent component of the gram-negative lipopolysaccharide (endotoxin) appears to be lipid A. Elucidation of the structure-function relationships of lipid A and the biochemical configurations required for endotoxicity makes possible the design of lipopolysaccharide antagonists and/or the production of poly- or monoclonal antibodies that may abrogate the biologic effects of endotoxin. The mechanisms of activity of lipopolysaccharide and the pathophysiologic events it triggers are now better understood than in the recent past. Lipid A triggers the release of mediators such as cachectin (tumor necrosis factor), thereby initiating a cascade of potentially lethal events. Although recent studies indicate no routine role for corticosteroids in gram-negative septic shock or acute respiratory distress syndrome, considerable progress has been made in the development of effective antibiotics. Recent studies of septicemia in neutropenic patients show survival rates significantly higher than those reported more than two decades ago.

Introduction

Lowell S. Young

Bloodstream infections with gram-negative bacteria have been one of the most important clinical challenges of the antimicrobial era [1]. Before 1945 enteric bacteria and organisms like *Pseudomonas aeruginosa* only infrequently were involved in bacteremia in normal hosts [2]. Today the incidence of nosocomial infections has increased dramatically as a result of aggressive treatments for cancer, organ transplantation, and the treatment of underlying diseases in elderly hospitalized patients. Gram-negative bacteria represent the most common cause of nosocomial infections. Some recent studies indicate that organisms such as skin-colonizing staphylococci are most common in bacteremia, but fatality ratios associated with gram-negative bacteria are far greater than those associated with skin-colonizing organisms such as *Staphylococcus epidermidis* [3].

Because gram-negative bacteria are part of the native microbial flora in humans, the normal host must have abundant means by which to resist systemic invasion. These include phagocytic cells, humoral antibodies, and nonspecific inflammatory mechanisms. Why certain gram-negative organisms and not others consistently cause disease in humans is poorly understood. *P. aeruginosa* and other clinically important organisms are thought to possess more virulence factors than the gram-negative organisms that cause serious disease only infrequently. Extensive research over the last two decades has concentrated on bacterial lipopolysaccharide (endotoxin) as a critical virulence factor in the pathophysiology of gram-negative infections, and much new information has become available since this subject was last reviewed [4]. Using modern physical chemistry techniques, researchers have delineated the structure of lipopolysaccharides and have identified the critical determinants of toxicity. However, the clinical problem of understanding the pathophysiology of gram-negative sepsis is far more complex.

Endotoxin, which appears to exert deleterious effects through a series of inflammatory pathways, probably repre-

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inserted into the 6′-position; this conclusion was based on ^{13}C nuclear magnetic resonance data (figure 1). Study of mutants deficient in KDO by means of fast-atom bombardment mass spectrometry, proton nuclear magnetic resonance, and chemical analyses determined the placement of the four β-hydroxymyristoyl moieties [10–14]. Other studies showed that laurate and myristate resides were the piggyback fatty acids on the nonreducing sugar of *E. coli* lipid A and that there was an additional palmitate on the reducing end of *Salmonella* lipid A [8, 14, 15].

Space-filling models in which the revised lipid A structure was used produced a planar conformation that (it was thought) would form a lipid bilayer in aqueous solutions [6]. This finding was consistent with lipid A's forming the outer leaflet of the outer membrane lipid bilayer.

Chemical synthesis of lipids according to the revised lipid A structure showed that the most biologically active lipids corresponded most closely to the structure shown in figure 1 [16–20]. In vitro and in vivo tests demonstrated that these activities were identical to those of naturally occurring compounds.

Elucidation of the biosynthetic pathway of lipid A, depicted in figure 2, also supported the structure proposed in figure 1 [11, 14, 21–31]. The first step in the biosynthetic pathway of endotoxin is fatty acylation of uridine diphosphate-*N*-acetylglucosamine (UDP-GlcNAc) [21, 29] to uridine-2,3-diacylglucosamine (UDP-2,3-diacyl-GlcN) [29]. This reaction is catalyzed by UDP-GlcNAc acyltransferase [23] and controlled by the *lpx*A gene [30]. A mutation in the *lpx*B gene (formerly known as *pgs*B) leads to accumulation of lipid X in *E. coli* [11, 30, 31]. This gene controls the production of lipid A disaccharide synthase [22], which directs condensation of lipid X and UDP-2,3-diacyl-GlcN to form the disaccharide. Next, a membrane-bound 4′-kinase phosphorylates the disaccharide to make the 1,4′-*bis*-phosphate product [23]. This is one product that accumulates in the KDO-deficient mutant of *Salmonella typhimurium* [14, 25]. The order of attachment of the KDO moieties [24], of additional polar moieties (4-amino-4-deoxy-L-arabinose at the 1-position or phosphoethanolamine at the 4′-position in *S. typhimurium* or pyrophosphate at the 1-position in *E. coli*), and of fatty acids (lauroyl, myristoyl, palmitoyl) to lipid A molecules remains to be established.

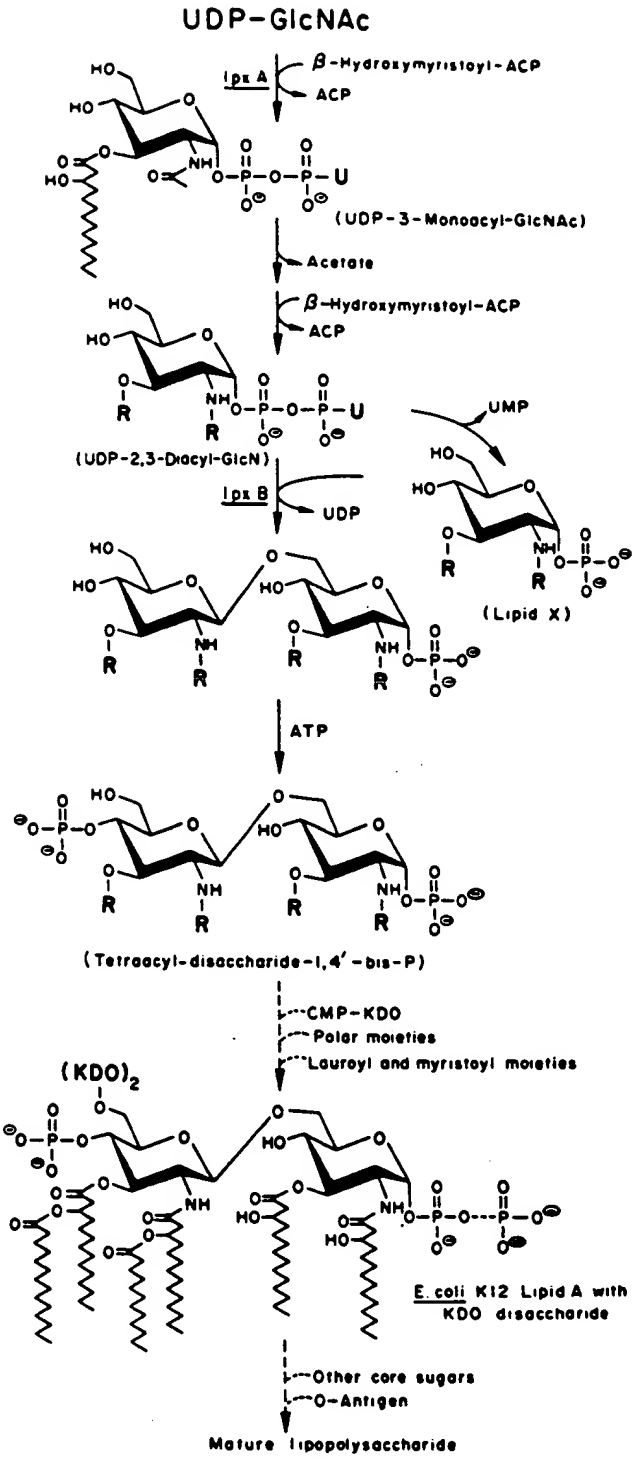

Figure 2. Postulated scheme for biosynthesis of lipid A in *E. coli* (see text for details). UDP-GlcNAc = uridine diphosphate-*N*-acetylglucosamine; UDP-2,3-diacyl-GlcN = uridine-2,3-diacylglucosamine; ACP = acyl carrier protein; UMP = uridine monophosphate; ATP = adenosine triphosphate; CMP = cytidine monophosphate; and R = β-hydroxymyristoyl moiety. Dashed bond indicates pyrophosphate residue found at the reducing end of some lipid A molecules. Reprinted with permission from [23].

Bi logic Activities of Lipid A Precursors and Derivatives

More than 40 mono- and disaccharide derivatives of lipid A have been studied and their biologic activities reported. The most important structure-function principles are described here.

For convenience, biologic activities of endotoxin can be separated into two groups: toxic (lethality, pyrogenicity, complement activation, disseminated intravascular coagulopathy) and immunologic (adjuvant activity, polyclonal B-cell stimula-

tion, macrophage activation, cytokine production). However, lipid A activities cannot be strictly separated. For example, lipid A-activated macrophages release interleukin 1 and tumor necrosis factor, which stimulate host defenses against bacterial infections and produce tumor necrosis while acting synergistically to cause profound shock and death. However, an understanding of relationships between the newly described chemical structure and the biologic activities of lipid A has led to the development of therapeutically useful lipid A-related compounds that either compete with the toxic effects of endotoxin or act primarily as immunostimulators with only minimal toxicity.

The presence of six fatty acids – the number on the lipid A of *E. coli* [6, 18] – appears to be optimal for the production of toxicity [18–20, 32–35]. The presence of a seventh fatty acid, palmitic acid, on the lipid A of *Salmonella* [12–15] was found to result in a lower level of activity than that observed for *E. coli* lipid A [34–36]. Similarly, lack of the β-acyloxyacyl groups reduced the lethal, pyrogenic, and local Shwartzman reaction activities of these disaccharides [37–40]. Phagocytic enzymes were also shown to remove β-acyloxyacyl groups [41, 42]. Nevertheless, these lipid A-like compounds retained significant toxicity, as measured by complement activation, enhancement of procoagulant activity, stimulation of tumor necrosis factor, and lethality [36–48, 43, 44]. Addition of fatty acids at the C6-position did not increase activity [45, 46]. The R forms of the stereoisomers tended to be somewhat more active than the L forms – a finding consistent with the natural form of lipid A, an R configuration; however, the presence of substitutions, such as hydroxyl or fatty acid groups, was the most important determinant of activity [32].

The removal of one phosphate group from these molecules greatly reduced their toxicity and activity [15, 36], and the removal of both left very little activity [36]. Lethal toxicity decreased more after removal of the phosphate group in the 1-position than after removal of the phosphate group in the 4′-position [47] (figure 1). This reduced toxicity may be attributable, in part, to increased hydrophobicity. In support of this concept, Kumazawa et al. [32] found that monosaccharides (lipid X-like compounds with four fatty acids) were less active than lipid X, perhaps because of increased hydrophobicity.

Biologic activity was also reduced by such small changes as the substitution of myristic acid for β-hydroxymyristic acid at the 3-position of monosaccharides and disaccharides with two and four fatty acids, respectively [32]. Addition of KDO to the monosaccharide did not appear to enhance activity [32]. However, this finding is controversial [48].

Finally, most lipid A precursors and derivatives caused clotting of the limulus amebocyte lysate. In general, disaccharides were more active than monosaccharides, and monosaccharides with three acyl groups were more active than those with two acyl groups [7, 18–20, 32, 35–38, 43, 49–51].

Several generalizations can be made from these studies, with some exceptions to each. Disaccharides appear to be both pyrogenic and immunologically active; in particular, these sugars stimulate tumor necrosis factor [37, 52–54]. An exception is GLA-60, which shows a negative Shwartzman reaction and lacks pyrogenicity but still stimulates tumor necrosis factor [32]. Monosaccharide precursors and derivatives of lipid A exhibit a much lower level of mitogenic activity than disaccharides. Monosaccharides also have much lower biotoxicity, showing only minimal pyrogenicity [55], no ability to stimulate tumor necrosis factor in normal mice [54], and only moderate ability to stimulate tumor necrosis factor in mice primed with *Propionibacterium acnes* [32].

Therapeutic Potential of Lipid A Precursors and Derivatives

As might be predicted from the biologic activities described, a disaccharide derivative of lipid A, monophosphoryl lipid A, has shown the greatest promise as an immunostimulator, whereas a monosaccharide derivative has demonstrated the greatest potential as an antiendotoxin [7, 55]. A working hypothesis is that pyrogenicity seems to correlate with immunoprotective activity. The release of mediators such as interferon, interleukin 1, and tumor necrosis factor may be essential to stimulate phagocytes and T cells that will protect the host from subsequent challenge.

Protection against challenge with endotoxin. As the structure of lipid A became more clearly defined, we began to study the activity of a number of its precursors and derivatives with the goal of defining structure-function relationships. We observed low-level toxicity of lipid X in sheep [56, 57] and mice [58]. Because lipid X is a subunit of lipid A, we tested its ability to block endotoxicity and found it to be protective [54]. As the time between endotoxin challenge and lipid X therapy increased, however, larger doses of lipid X were needed and effectiveness decreased. Of note, we found that high doses of lipopolysaccharide overcame the protective activity of lipid X and that this effect was not reversed by the administration of higher doses of lipid X (author's unpublished data). These observations suggested that there is a window of protection by lipid X against endotoxicity. Similarly, pretreatment with lipid X protected galactosamine-sensitized mice from endotoxin [59]. The failure of lipid X to protect these mice when given after endotoxin challenge may be attributable to the steep endotoxin dose-response mortality curve; the narrow window of protection may have been missed.

Pretreatment with lipid X also decreased mortality among sheep challenged with endotoxin [55]. These animals demonstrated significantly lower pulmonary artery pressures during both early- and late-phase responses to endotoxin challenge. It was interesting that lipid X did not prevent endotoxin-induced neutropenia, nor did it block the systemic hypotensive response to endotoxin. The one animal not protected by lipid X received material that was contaminated with

lipid Y, which is toxic to sheep [57]. Thus lipid X protected both sheep and mice from the lethal effect of endotoxin.

A monophosphoryl derivative of lipid A also protected mice against challenge with endotoxin [60]. This compound is *Salmonella minnesota* Re lipid A without KDO moieties and with the phosphate group removed from the 1-position—hence, monophosphoryl lipid A [7, 47, 61, 62]. When this lipid A derivative was given 1 day before challenge, the LD_{50} of endotoxin increased by approximately fivefold [60].

Recently, a nontoxic lipid A was harvested from *Rhodopseudomonas sphaeroides* and its complete structure established [63]. Although this is a disaccharide hexaacyl compound, it blocked the production of tumor necrosis factor by RAW 264.7 macrophages [63]. *R. sphaeroides* lipid A has two major structural differences from the lipid A of both *E. coli* and *Salmonella*: the presence of a 3-ketodecanoate instead of a 3-hydroxytetradecanoate at the 2-position and a Δ^7-tetradecanoate instead of a tetradecanoate in acyloxyacyl linkage at the 2'-position. This study confirmed earlier work showing nontoxicity of complete *R. sphaeroides* endotoxin [64] and suggested that modification of the acyl chains may be a fruitful source of endotoxin antagonists for trials in experimental animals.

Protection against bacterial challenge. Lipid X also reduced mortality among *E. coli*–challenged mice [65]. Alone, lipid X slightly prolonged life [65]. The combination of lipid X with ticarcillin enhanced the survival rate significantly more than did ticarcillin alone. Specifically, the survival rate increased by two- to fourfold after treatment with lipid X and ticarcillin over a broad range of doses. This effect continued for 3 days after the administration of ticarcillin was stopped. When administered with lipid X, the dose of ticarcillin necessary to protect 50% of mice from death was reduced by two- to fivefold. Pretreatment with lipid X was not necessary for enhancement of the survival rate: 16 (94%) of 17 infected and toxic mice that were treated with lipid X and ticarcillin 6 hours after *E. coli* challenge survived, whereas only 30 (68%) of 44 controls treated with ticarcillin alone survived ($P < .0001$) [65]. These data suggest that lipid X may inhibit some endotoxic activity released by ticarcillin therapy. Alternatively, lipid X may stimulate host defenses. This alternative seems less likely, however, because highly purified lipid X alone showed protective activity within 18 hours (a response that seems quite rapid for the production of antibodies [65]) and because both highly purified biologic lipid X and synthetic lipid X were very weak mitogens and releasers of cytokines [54, 66], whereas less purified lipid X caused greater macrophage activation [52, 67].

Mechanisms f Protecti n

How lipid X may exert antiendotoxic activity is not known. The original impetus for the testing of lipid X for protective efficacy was the hypothesis that it might compete with endotoxin for a membrane receptor, but further data make that possibility less likely. First, lipid X does not compete with endotoxin in a simple antagonist-agonist pattern; i.e., the ratio of agonist to antagonist is not constant. In fact, lipid X does not compete with endotoxin or lipid A over a broad range of concentrations but only within a narrow window. Notably, the effective range of protection for lipid X appears to coincide with endotoxin levels observed in gram-negative sepsis. Second, lipid X does not compete with endotoxin or lipid IV_A, a lipid A precursor, for a membrane receptor on macrophages [68]. Hence, lipid X probably acts on postreceptor signal transduction or secretory systems. In support of this concept, we have preliminary data (obtained in vivo and in vitro in RAW 264.7 cells) that lipid X reduces endotoxin-induced production of tumor necrosis factor (author's unpublished data). This finding is consistent with data from Chia et al. [59], who showed reduced production of tumor necrosis factor by mice peritoneal macrophages treated in vitro with endotoxin and lipid X alone or in combination.

At the cellular level, neither lipid X nor monophosphoryl lipid A inhibited endotoxin-mediated adherence of neutrophils to human umbilical veins [69], but the diphosphoryl tetraacyl lipid A precursor did inhibit neutrophil adherence [70]. This observation suggests that these lipids do not mediate protection by inhibiting neutrophil adherence. In other studies lipid X (1) blocked endotoxin-mediated priming of human neutrophils [71, 72]; (2) inhibited endotoxin-induced macrophage procoagulant activity, both directly at the macrophage level and indirectly via the T cell [73, 74]; (3) decreased the mitogenic response induced by *S. minnesota* Re endotoxin in mouse splenocytes [66]; and (4) reduced lipid IV_A–mediated expression of IgM on murine B cell lymphoma 70Z/3 cells [75]. Lipid X also reduced the release of serotonin from lipid A– and phorbol myristate acetate–stimulated platelets but not from thrombin-stimulated platelets [76]. This reduction occurred in the absence of cyclic AMP stimulation. Lipid X blocked the activation of protein kinase C in platelets but not in cultured endothelial cells [77]. Finally, lipid X blocked lipid A precursor–mediated activation of complement [37]. Thus lipid X exerts an antiendotoxic effect on monocytes, peritoneal macrophages, T cells, B cells, platelets, and neutrophils, but probably not at the cell-receptor level. More complete knowledge of endotoxin-mediated events may be essential to an understanding of the mechanisms of lipid X activity at the subcellular level.

In contrast to lipid X, monophosphoryl lipid A exhibited many immunostimulating properties that may protect the host from bacterial invasion by activating host defenses [47, 61, 62]. Monophosphoryl lipid A from *S. minnesota* R595 reduced mortality when injected into mice from 1 day before to 2 days after challenge with 1 LD_{65} to 1 LD_{100} of *E. coli* or *S. epidermidis* [78]. This lipid A derivative also enhanced tumor lysis (in vitro and in vivo), antibody production, and antiviral activities in macrophages [47, 61, 62, 66, 79]. Thus

treatment with monophosphoryl lipid A activates macrophages [47, 61, 62], releases cytokines [60], and inactivates suppressor T cells [80]. The mechanisms underlying these activities are unknown.

Conclusions

Lipid X and monophosphoryl lipid A should probably be viewed as early prototypes for compounds to be used as immunostimulants and antiendotoxic chemotherapeutic agents. Development of these lipid A precursors has followed from an understanding of the structure of lipid A. Because endotoxin is one of the most active compounds in a substantial number of biologic systems, the discovery of several very active and therapeutically valuable agents is likely. The potential for discovery of such agents will be further enhanced when the subcellular mechanisms of lipid A activities are more completely understood.

Cachectin (Tumor Necrosis Factor) in the Pathogenesis of Gram-Negative Shock

Bruce Beutler

Septic syndrome and its many components have been exhaustively described. It is well known that responses to the administration of bacterial lipopolysaccharides closely mimic sepsis in animal models. It is perhaps less well appreciated that lipopolysaccharides, whatever their effects on tissues of sensitive animals, do not directly elicit shock or organ damage. The elegant studies of Michalck et al. [81] showed that lymphoreticular cells confer the lethal effect of lipopolysaccharide. Endotoxin-resistant mice of the C3H/HeJ strain were rendered sensitive to the lethal effect of endotoxin by adoptive transfer of marrow from sensitive histocompatible mice. Conversely, endotoxin-sensitive animals were rendered endotoxin resistant after receiving irradiation and marrow transplanted from C3H/HeJ mice. Thus for the past decade a cell or factor of hematopoietic origin has been considered responsible for the mediation of endotoxicity.

Other diseases also appear to be host mediated. Wasting diathesis, which accompanies certain restricted tumors or parasitic infections such as trypanosomiasis, has long been thought to depend on host factors released in response to an invasive agent. Other paraneoplastic problems and most (if not all) inflammatory problems probably are also host mediated. Molecular analysis of biologically important cytokines and isolation of genes encoding for their production now permit close scrutiny of the once-nebulous factors regulating host-mediated diseases. Molecular analysis has been used to isolate and purify these factors, to determine their primary structures, and to gauge their effects in many well-controlled systems.

One of the most important proinflammatory mediators is cachectin, or tumor necrosis factor. This protein has an intriguing history. It was independently isolated by investigators interested in two very different effects of lipopolysaccharide. As cachectin, the protein was purified as the agent responsible for the hypertriglyceridemia observed in endotoxic shock [82, 83]. As tumor necrosis factor, it was purified as the agent responsible for inducing the hemorrhagic necrosis observed after endotoxic shock in transplantable tumors of mice [84, 85]. When the identity of cachectin and tumor necrosis factor as a single mediator was established, the likelihood of its involvement in many diseases affecting humans and animals became apparent.

The Search for a Mediator of Wasting and Shock

Rouzer and Cerami [82] observed that rabbits infected with African trypanosomes often exhibited a striking degree of hypertriglyceridemia despite severe anorexia and wasting. This metabolic derangement was traced to a clearing defect: lipoprotein lipase, the enzyme that normally removes triglycerides from the circulation, was systemically suppressed in these animals. Suspecting that the suppression of lipoprotein lipase was an active process mediated by the immune system, Kawakami and Cerami [86] devised a second model system to identify hormones capable of such suppression. They showed that endotoxin-induced macrophages secreted a factor that suppressed the expression of lipoprotein lipase in the fatty tissues of endotoxin-unresponsive C3H/HeJ mice. This factor, termed *cachectin*, was produced in response to many microbial agents but most abundantly in response to lipopolysaccharide. Cachectin was purified to homogeneity on the basis of its ability to suppress the expression of lipoprotein lipase in cultured adipocytes of the 3T3-L1 cell line. The purified hormone was a protein with a subunit size of ~17.5 kD. It was secreted in large quantities by activated macrophages, comprising up to 2% of the total secretory product of these cells. Cachectin was found to bind to a high-affinity receptor on the surface of most cultured mammalian cells and of many tissues studied in vitro.

In further studies we observed that cachectin induced many metabolic responses in cultured adipocytes, suppressing the biosynthesis of several fat-specific proteins at the transcriptional level [87]. We also found that cachectin entered the circulation after its production in vivo and was cleared primarily by binding to its plasma membrane receptor [88]. The in vivo half-life of the hormone in mice was ~6 minutes. The active protein appeared to be produced only briefly after exposure of the macrophage population to lipopolysaccharide. Thus a mechanism for the suppression of biosynthesis appeared to preclude unfettered production of the protein.

When the *N*-terminal amino acid sequence of cachectin was determined, a striking degree of homology to the human protein designated *tumor necrosis factor* was noted. As has been mentioned, tumor necrosis factor has been isolated as a medi-

ator responsible for the induction of hemorrhagic necrosis of tumors in vivo [84] and for cytolysis of some transformed cells in vitro [85]. In addition, cachectin and tumor necrosis factor showed identical patterns of activity in reciprocal bioassays and were immunologically related. It was therefore suspected that cachectin represented the murine homologue of human tumor necrosis factor. This supposition was confirmed by molecular cloning studies in which the cDNA sequence of human tumor necrosis factor was used in the design of a probe to isolate the cDNA-encoding mouse tumor necrosis factor [89]. The mouse cDNA sequence exactly predicted the cachectin sequence. For convenience, the term *cachectin* in the following discussion encompasses both cachectin and tumor necrosis factor.

Structure and Biosynthetic Control of Cachectin

Cachectin is a trimeric protein made up of equal subunits [90, 91], each with a molecular mass of ~17.5 kD [90, 91]. Each subunit is composed principally of β-pleated sheets [91-93]. Cachectin is initially synthesized as a prohormone containing 75 amino acids, which are appended at the amino terminus in the human form of the protein. The propeptide contains a hydrophobic region that anchors the protein in a lipid bilayer. This propeptide is subsequently cleaved at two or more sites to liberate the mature subunit. The extensive conservation of the propeptide portion of the molecule suggests that it may have other functions as well.

The process by which lipopolysaccharide effects biosynthesis of cachectin has received considerable attention [94]. Upon endotoxic activation of the cell, the cachectin protein is synthesized de novo and efficiently transported from the cell. Cachectin production is controlled at several levels. Although the signal transduced by lipopolysaccharide remains to be determined, lipopolysaccharide clearly enhances transcription of the cachectin gene and augments synthesis posttranscriptionally. The latter effect may conceivably involve stabilization of cachectin mRNA as well as increased efficiency at the translational level.

The synthesis of cachectin was strongly inhibited when glucocorticoid hormones were applied to macrophages before endotoxin activation [94]. Although inhibition depended on the suppression of transcription and translation, the mechanisms mediating these suppressive effects remain unclear. The inhibitory effects of glucocorticoids may partly explain induction of the endotoxin-refractory state observed in experimental animals. The finding that glucocorticoids were effective only if administered in advance of challenge with endotoxin may explain their limited efficacy in the treatment of septic shock in the clinical setting.

Cachectin is structurally related to the hormone lymphotoxin [95-97], which is also known as tumor necrosis factor β and which arose—probably early in vertebrate evolution—from a tandem duplication event. At the level of protein, lymphotoxin has ~30% homology to cachectin. It is produced by lymphocytes (rather than macrophages) in response to lymphocyte-activating stimuli. It appears to bind to the same plasma membrane receptor as cachectin and evokes a similar spectrum of biologic responses. However, lymphotoxin is not produced in response to lipopolysaccharide and probably plays a minimal role in the pathophysiology of sepsis.

Biologic Effects of Cachectin

Cachectin elicits strikingly different biologic responses, depending upon dose and schedule of administration. Because at least two very different effects of lipopolysaccharide were mediated by cachectin synthesis, it was initially suspected that cachectin might be important in the pathogenesis of endotoxic shock. Indeed, passive immunization against cachectin significantly protects animals against the effects of subsequent challenge with endotoxin. This observation, made initially in experiments with mice [98], has been extended to include primates [99] and rabbits [100]. Moreover, both mono- and polyclonal antibodies have been studied. When cachectin was administered in large doses to rats [101], dogs [102], or primates [99], it evoked a fulminant shock syndrome characterized by metabolic acidosis, hypotension, interstitial pneumonitis, acute renal tubular necrosis, and mesenteric ischemia sometimes leading to infarction of large segments of the bowel. Changes observed at necropsy were, for the most part, similar to those produced by endotoxic poisoning. Thus cachectin almost certainly can and does mediate a substantial proportion of endotoxin-induced injury.

An entirely different set of responses was observed in animals chronically exposed to lower concentrations of the hormone. To test our suggestion [83] that cachectin could, if chronically produced, lead to anorexia and wasting, Oliff and colleagues [103] developed a novel system for chronic administration of cytokines. By transfecting Chinese hamster ovary cells with a vector that caused continuous secretion of recombinant human cachectin and then inoculating these cells into the hind limb of nude mice, these investigators produced small, nonmetastatic, cachectin-secreting tumors. These tumors were associated with wasting diathesis, whereas control tumors produced with an empty vector were associated with normal weight gain and growth. Although the mechanisms eliciting these effects remain to be determined, the exciting implication is that a single mediator may cause cachexia as well as a syndrome closely related to septic shock. Such strikingly different pathologies would not previously have been thought to have a common mediator.

Role of Cachectin in Shock, Cachexia, and Other Conditions

The measurement of plasma levels of cachectin has usually been technically difficult because the hormone is produced only briefly in response to lipopolysaccharide and is cleared rapidly. However, Waage et al. [104] recently showed that markedly elevated levels of cachectin in plasma correlated with a fatal outcome in patients with meningococcal septicemia. Balkwill et al. [105] found elevated concentrations of cachectin in the plasma of patients with neoplastic diseases; however, levels were generally lower than those in experimental sepsis, and no correlation was established between the degree of weight loss and the cachectin level. Socher et al. [106] found no detectable cachectin in the circulation of patients with cancer-associated cachexia stemming from restricted neoplasms whose catabolic effect was presumed to be caused by a humoral mechanism. The ability of cachectin to cause wasting and the absence of circulating cachectin in patients with naturally occurring cachexia have not been reconciled. However, chronic exposure to cachectin at levels undetectable by current assays may cause cachexia.

Piguet et al. [107, 108] recently suggested that cachectin may be an important mediator of the acute phase of graft-vs.-host disease. In two studies they suggested that the hormone is essential for granuloma formation in mycobacterial infection. Grau et al. [109] proposed that the protein plays an essential role in the development of murine cerebral malaria. The same situation probably obtains in humans.

Garrett et al. [110] recently demonstrated that human myeloma cell lines often secrete lymphotoxin. They showed that this protein stimulates resorption of bone through an osteoclast activity factor–like effect. Thus lymphotoxin may be responsible for the hyperkalemia and bone resorption observed in multiple myeloma.

Cachectin in Clinical Practice

Like the inflammatory response itself, cachectin undoubtedly evolved to check the spread of disease. Supporting this view, for example, is the observation that the administration of cachectin to C3H/HeJ mice, which fail to produce the protein in response to lipopolysaccharide, protects the animals against *E. coli* sepsis [111]. Yet, when produced in excess or for an inappropriately long time, the hormone has obvious deleterious effects. Thus a delicate balance exists. Failure to block the inherent toxic effects of cachectin may lead to the death of the host, whereas inappropriate inhibition of cachectin synthesis or activity may favor progression of the primary disease. Considerable clinical experience will be required to determine the precise circumstances under which therapeutic intervention should be attempted. Means of inducing or preventing the production of cachectin and of administering large quantities of this hormone are currently available. In the future, selective antagonism of cachectin may be feasible. Clinical testing may ultimately reveal benefits of inhibiting the activity or synthesis of cachectin in the management of sepsis.

Potential for Immunotherapy in Gram-Negative Bacillary Infections

William R. McCabe

Infections caused by gram-negative bacilli have remained one of the major therapeutic problems in infectious diseases for 25 years. Serious gram-negative bacillary infections are characterized by both a high prevalence and a high mortality. Estimates of the number of episodes of bacteremia involving gram-negative bacilli annually in the United States range from 71,000 to >300,000 [112–114]. Similarly, gram-negative bacilli are believed to be the most frequent cause of nosocomial pneumonia. Neither the introduction of numerous new antibiotics highly active against gram-negative bacilli nor the institution of vigorous hospital infection-control measures appears to have materially reduced the number of deaths caused by these infections [113].

This continuing problem has prompted some investigators to examine therapeutic and preventive measures other than antibiotic administration and infection control. Although immunization with gram-negative bacilli might be possible, the characteristics of gram-negative infections, coupled with prior experience with immunization, appear to preclude this approach. Historically, immunization (active and passive) has proven effective against several bacterial infections, but this response has generally been limited to antitoxic immunity or type-specific immunity to a limited number of serologic types.

Studies of gram-negative bacillary bacteremia have identified multiple species as etiologic agents, with *E. coli* and *Klebsiella pneumoniae* the most frequent isolates [114]. Multiple serotypes of these two species have been isolated from the blood. In one hospital serologic typing identified 31 O antigen types, 24 unclassifiable types, and six rough strains among 149 consecutive blood culture isolates of *E. coli* and 16 capsular types among 30 isolates of *K. pneumoniae* [114]. This large number of species and serotypes responsible for gram-negative septicemia in a single hospital would appear to render type-specific active or passive immunization ineffective for the treatment or prevention of gram-negative bacillary infections.

An alternative to type-specific immunization was suggested after efforts by bacterial geneticists and biochemists had clearly defined the structure and chemical composition of the lipopolysaccharide in the cell wall of gram-negative bacilli. These studies demonstrated that the outer portion of the lipopolysaccharide molecule was composed of high-molecular-weight

Table 1. Protective effect of passive transfer of serum from rabbits immunized with S and R strains of *S. minnesota* against challenge of mice with an ordinarily lethal dose of *K. pneumoniae* or *M. morganii*.

Type of treatment before challenge	No. of mice surviving challenge	
	K. pneumoniae (n = 22)	*M. morganii* (n = 20)
Serum from control rabbits treated with		
Saline	5*	2†
Nonimmune rabbit serum	6*	2†
Serum from rabbits immunized with *S. minnesota* strain		
S218	6	8
Ra	10	2
Rb	3	0
Rc	6	4
Rd_1	7	6
Rd_2	12	6
Re	18*	20†

NOTE. Table is adapted with permission from [5].
* χ^2 = 13.8 and $P < .001$ vs. *S. minnesota* Re.
† χ^2 = 45 and $P < .001$ vs. *S. minnesota* Re.

carbohydrate polymers whose sugar composition and linkages were unique for each gram-negative serotype. In contrast, the inner core of almost all gram-negative bacilli contained identical sugars linked terminally to lipid A [115]. The virtually identical composition of the core portion of lipopolysaccharides suggested that shared or common epitopes might be exposed on the bacterial surface and that antibody to these common antigens might enhance resistance to gram-negative bacilli.

Studies in Animal Models

Two groups of investigators began to evaluate this possibility almost simultaneously by immunizing animals with rough (R) mutants whose immunodeterminant terminal sugar was present in the lipopolysaccharide core of all gram-negative bacilli [5, 116–121]. Initial studies in our laboratory involved immunization of rabbits with the parental smooth (S) *S. minnesota* strain S218 or its R mutants of the Ra, Rb, Rc, Rd_1, Rd_2, and Re chemotypes [5]. Antiserum to each strain was administered intravenously to mice that were then challenged with 100 LD_{50} of viable *K. pneumoniae* or with 10 LD_{50} of *Morganella morganii*. Antiserum to the Re mutant was considerably more protective than antiserum to any other strain, resulting in survival rates of ≥85% (table 1).

Active immunization with the Re mutant also protected mice against lethal intravenous challenge with heterologous bacterial strains [5]. Protective activity induced by the Re mutant was compared with type-specific immunity; specifically, the number of *K. pneumoniae* or *M. morganii* organisms constituting the LD_{50} for control mice was compared with that constituting the LD_{50} for mice immunized with the Re mutant or with the homologous challenge strain (table 2). As anticipated, type-specific immunization was considerably more effective than immunization with the Re mutant. However, the latter did provide significant protection by increasing the number of bacteria required for an LD_{50} by 150-fold for *K. pneumoniae* and by 15-fold for *M. morganii*.

Active immunization with the Re mutant also protected rabbits with granulocytopenia induced by nitrogen mustard against bloodstream infection with *Enterobacter aerogenes* and two serotypes of *E. coli* [116]. However, immunization with lipid A from the Re mutant afforded no protection. Later

Table 2. Comparison of type-specific protection with that induced in mice by immunization with the Re mutant of *S. minnesota*.

Challenge strain and dose	No. of mice surviving challenge after immunization with indicated agent*				
	Saline	*P. aeruginosa*	*S. minnesota* (Re mutant)	*K. pneumoniae*	*M. morganii*
K. pneumoniae†					
1×10^6	1	0	2	10	NA
1×10^5	1	2	5	10	NA
1×10^4	1	2	7	10	NA
1×10^3	4	3	7	10	NA
1×10^2	4	4	10	10	NA
M. morganii‡					
1.5×10^8	0	0	2	NA	10
1.5×10^7	0	3	10	NA	10
1.5×10^6	10	10	10	NA	10

NOTE. Table is adapted with permission from [5].
* Data shown reflect survival 72 hours after challenge. There were 10 mice in each group. NA = data not available.
† The LD_{50} of *K. pneumoniae* was 2.7×10^2, 3.3×10^2, 4.4×10^4, and $>1.0 \times 10^6$ after administration of saline, *P. aeruginosa*, *S. minnesota*, and *K. pneumoniae*, respectively. (No data are available for administration of *M. morganii*.)
‡ The LD_{50} of *M. morganii* was 4.7×10^6, 7.8×10^6, 6.3×10^7, and $>1.5 \times 10^8$ after administration of saline, *P. aeruginosa*, *S. minnesota*, and *M. morganii*, respectively. (No data are available for administration of *K. pneumoniae*.)

studies demonstrated that passively administered rabbit antisera to the Re mutant and to the J5 mutant of *E. coli* also protected mice against lethal challenge with heterologous lipopolysaccharide [117]. The protective effect of Re antisera against lethal challenge with heterologous gram-negative bacilli and endotoxin was also shown to be removed by immunoabsorption with the Re lipopolysaccharide [5, 117].

Similar studies were carried out during the same period by another group, led by the late Dr. A. I. Braude [118–120]. These investigators used a different R mutant, the J5 mutant of *E. coli*. The lipopolysaccharides of both the Re mutant and the J5 mutant contain lipid A linked to ketodeoxyoctonate, but the J5 mutant also contains heptose and glucose. Extensive studies by Braude's group demonstrated that active and passive immunization with the J5 mutant protected mice and granulocytopenic rabbits against lethal challenge with several heterologous gram-negative bacilli. This group also established that antisera to the J5 mutant protected against both local and generalized Shwartzman reactions and against the lethality of heterologous endotoxin.

Both groups [5, 116–121] used immunofluorescence assays and ELISAs to demonstrate that antisera to the respective mutants reacted serologically with whole gram-negative bacilli and lipopolysaccharide. Other studies have shown that the immunologic reactivity of whole gram-negative bacilli or endotoxin with antisera to R mutants is most readily apparent with organisms in the early logarithmic phase (5 hours) of growth [122]. This observation suggests that immunodeterminants shared with R mutants are most accessible in S gram-negative bacilli during the early phase of bacterial growth. It may also explain why the protective activity of R antisera is most easily demonstrated against challenge with small inocula of gram-negative bacilli requiring multiple cycles of replication for lethality.

These reports of protective activity induced by immunization with R mutants stimulated similar studies by many other investigators. More than half of these studies – too numerous to describe in detail here – confirmed the immunologic cross-reactivity and protective activity of antibody to R mutants [123–132], while the remainder failed to confirm either cross-reactivity or protective activity of antisera to R mutants [133–139]. Some of the divergent results appeared to reflect the use of less-than-optimal assay methods. Another problem (reported in several studies) may have been the use of animal models requiring large bacterial inocula for lethality; the same investigators readily demonstrated protective activity of R antisera in experiments with more virulent challenge strains that produce lethal infections with smaller inocula [128, 130, 140].

Studies in Volunteers

Studies of experimental animals in which protective effects of immunization with the Re and J5 mutants were evident led to studies of the immunization of humans against lethal challenge with viable bacteria and lipopolysaccharide. We used vaccines prepared from the Re mutant to immunize 122 volunteers [141]. Immunization induced an antibody response to Re lipopolysaccharide and the development of protective activity in postimmunization sera. These findings were confirmed by comparison of the ability of sera obtained from volunteers before and after immunization to protect mice against an ordinarily lethal challenge with viable *K. pneumoniae*, *M. morganii*, or lipopolysaccharide from *Salmonella typhi*. Because high levels of type-specific antibody have much greater protective activity than do high levels of antibody to R mutants [5], we could not demonstrate the induction of protective activity after immunization with the Re mutant in volunteers who had type-specific antibody to the two assay strains. Among immunized volunteers without protective preimmunization sera, we observed increased postimmunization protective activity against lethal challenge with *K. pneumoniae* (67%), *M. morganii* (77%), or lipopolysaccharide from *S. typhi* (97%) (figure 3).

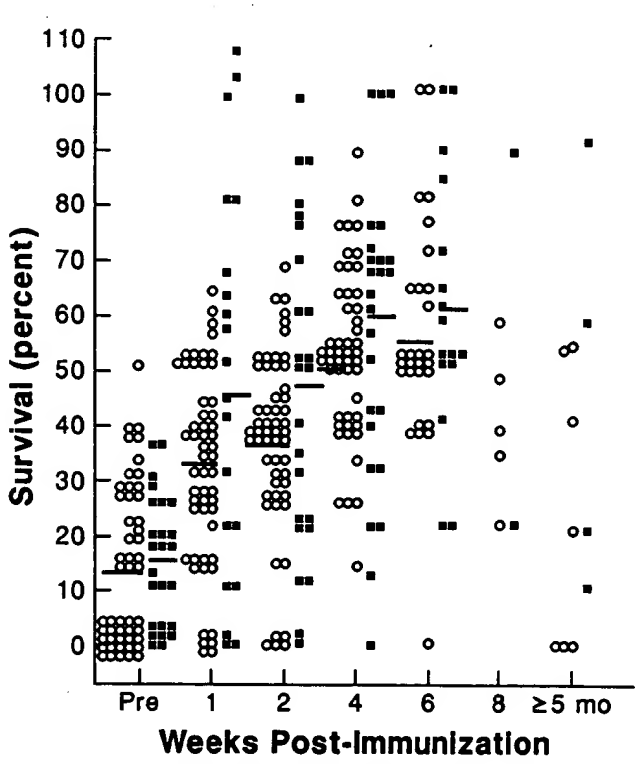

Figure 3. Summary of studies of mouse protection against endotoxic and viable bacterial challenge by the administration of serum from recipients of the Re mutant vaccine. Protection is expressed as the percentage of survivors among the eight to 10 mice receiving serum obtained from volunteers at the times shown. Mice received serum 1 hour before challenge with 50 LD_{50} of *Salmonella typhi* lipopolysaccharide (*open circles*) or with either 100 LD_{50} of whole, viable *K. pneumoniae* or 10 LD_{50} of whole, viable *M. morganii* (*solid squares*). Horizontal lines denote mean values. Reprinted with permission from [141].

Ziegler et al. [142] used antisera to the J5 mutant with impressive results in a clinical trial of therapy for gram-negative bacteremia. Units of plasma obtained before and after immunization with the J5 mutant were collected and used with appropriate antibiotics in this randomized trial including 191 episodes of gram-negative bacteremia. Fatality rates among patients receiving J5 antisera were lower than those among bacteremic patients receiving preimmunization sera (24% vs. 38%; $P = .04$). Even among bacteremic patients with profound shock, treatment with the J5 antisera resulted in substantially lower fatality rates than did treatment with preimmunization sera ($P = .009$) [142].

The effectiveness of active and passive immunization with R mutants, shown both in experimental animals and in clinical trials, suggests that such therapy would be of considerable clinical utility and commercial value. Regrettably, however, neither vaccines nor antisera are currently available for clinical use. This lack reflects the paramount importance of the IgM antibody in postimmunization R antisera in the mediation of protective activity and the problems involved in the isolation and purification of commercially useful amounts of IgM antibody suitable for human use. Studies in our laboratory of fractionated protective serum obtained from rabbits and humans after immunization with the Re mutant of *S. minnesota* demonstrated that the protective activity was mediated solely by IgM antibody and that IgG antibody exhibited no protective activity [141]. We have attempted to circumvent the problems of IgM antibody production by the development of a component vaccine consisting of modified Re lipopolysaccharide coupled to a protein carrier that is capable of inducing an anamnestic response after a booster injection in which IgG with protective activity is elicited (author's unpublished data). Other attempts to overcome these difficulties have included the preparation of murine and human monoclonal antibodies with demonstrable protective activities [143–150]. Two monoclonal antibodies—a murine IgM monoclonal antibody (E5; XOMA, Berkeley, CA) and a human IgM monoclonal antibody (Centoxin, HA-1A; Centocor, Malvern, PA) reactive with the lipid A portion of lipopolysaccharide—have undergone clinical trials. Results of these trials have been described briefly in abstracts [151, 152] and presented at national meetings, but information sufficient for critical, in-depth analysis is not yet available. The brief descriptions and oral presentations do indicate that the human monoclonal antibody significantly increases survival rates among patients with gram-negative bacteremia (with or without septic shock) [152]. Complete delineation of the clinical efficacy of monoclonal antibodies to lipid A in the treatment of gram-negative bacillary bacteremia and septic shock will require careful analysis of the results of these clinical trials and will probably entail additional clinical trials as well.

Steroidal and Nonsteroidal Pharmacologic Intervention in Septic Shock

John N. Sheagren

The syndrome of severe sepsis is now better described than ever before as a result of data collected from prospective, randomized studies of severely septic patients treated with glucocorticoids [153, 154]. Severe sepsis is classically typified by abrupt onset of fever, shaking chills, confusion, dyspnea, and tachycardia. The production of a variety of mediators results in vasodilation (with decreased systemic vascular resistance), compensation for which is provided by increased heart rate and cardiac output. Initial changes in orthostatic blood pressure are followed by refractory hypotension (due to capillary leakage with decreased relative intravascular volume and to progressive "septic" cardiomyopathy) and ultimately by signs and symptoms of the failure of multiple organ systems [155, 156]. Accompanying this syndrome of shock and organ failure is a progressive, severe metabolic acidosis with elevated levels of lactic acid. Although viable bacteria initiate these clinical sequelae, fewer than half of the patients presenting with this syndrome actually have bacteria isolated from the bloodstream [153, 154, 156].

Components of the microbial cell wall can trigger the syndrome of severe sepsis in both humans and experimental animals. These components include the endotoxin in gram-negative microbes, the teichoic acid–peptidoglycan complex in gram-positive microbes, and the yeast cell-wall polysaccharides. Although some systemic damage during the septic syndrome clearly results from the spread of viable microbes, most systemic damage is secondary to the triggering of host-originated mediators, many of which, paradoxically, are involved in local host defenses. When triggered by intravascular microbes or their products, these mediators may become counterproductive to the host, substantially exacerbating systemic damage. The rationale for antiinflammatory treatment of sepsis is to interfere with damaging mediators (including some products of arachidonic acid) while controlling local and systemic bacterial proliferation with antibiotics. The potential for minimizing systemic damage by treatment with steroidal agents (glucocorticoids) or nonsteroidal antiinflammatory agents is discussed below.

Effects of Antiinflammatory Agents on Potentially Damaging Mediators

Figure 4 describes the pathophysiology of complications of severe sepsis, and figure 5 indicates therapeutic options for countering these complications [155].

Glucocorticoids. In a wide variety of animal models, administration of glucocorticoids almost completely obviated the septic shock and multiple organ system failure produced

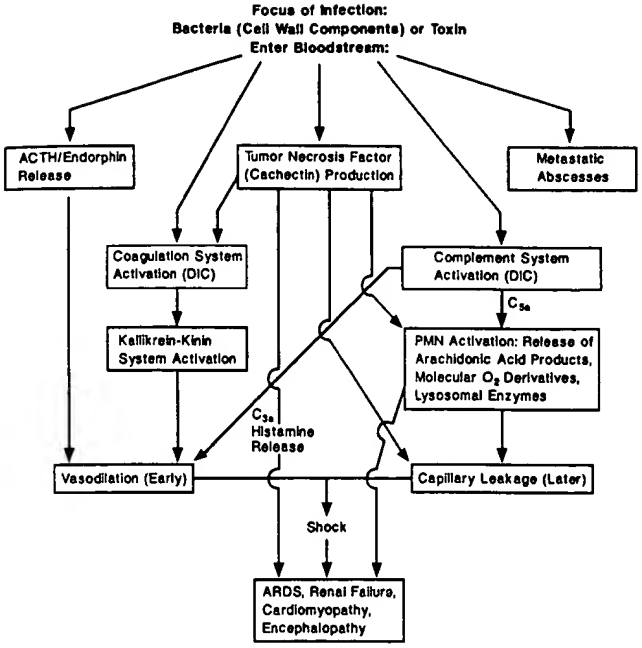

Figure 4. Pathophysiology of complications of severe sepsis. ACTH = adrenocorticotropic hormone; DIC = disseminated intravascular coagulation; PMN = polymorphonuclear leukocytes; and ARDS = adult respiratory distress syndrome. Adapted with permission from [155].

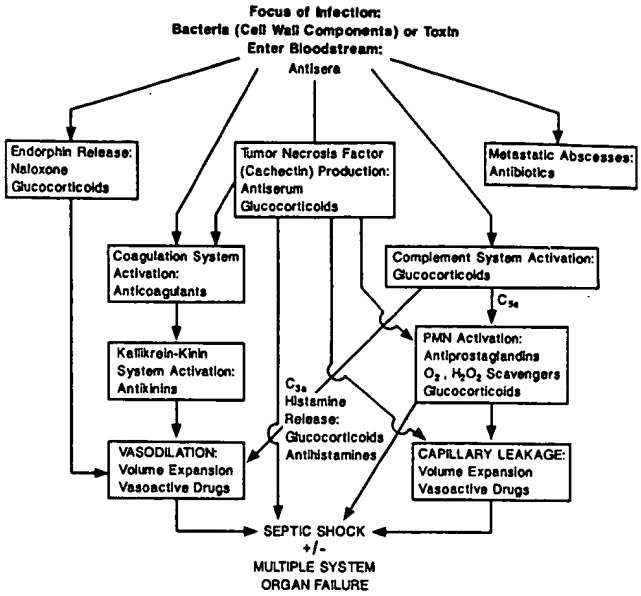

Figure 5. Therapeutic options for complications of severe sepsis. PMN = polymorphonuclear leukocytes. Adapted with permission from [155].

by purified bacterial endotoxins or viable gram-negative bacteria; these findings have been extensively reviewed elsewhere [157]. However, the baboon model of septic shock (the best-studied animal model) is worth considering in some detail. This model has been thoroughly described by Hinshaw and colleagues [158–160]. Lightly anesthetized baboons exhibited all of the classic hemodynamic characteristics of septic shock and ultimately died of multiple organ system failure. When a large dose of glucocorticoids was administered systemically at or shortly before infusion of 1 LD_{100} of *E. coli*, the expected 100% mortality was completely reversed: all animals survived and were completely normal when killed days or weeks later [158]. When the infusion of glucocorticoids was delayed until 2 hours after the infusion of gram-negative microbes, ~20% of the animals died and the rest were critically ill for several days [159]. When the infusion of glucocorticoids was delayed until 4 hours after the infusion of *E. coli*, almost half of the animals died [160]. As shown in figure 5, glucocorticoids theoretically may benefit patients with severe sepsis at multiple points in the evolution of the syndrome.

Nonsteroidal agents. The administration of nonsteroidal antiinflammatory agents, including both cyclooxygenase and thromboxane synthetase inhibitors, has also been tested in animal models of septic shock. The oldest and best-studied cyclooxygenase inhibitor is aspirin, which irreversibly blocks the cyclooxygenase system [161]. Studies many years ago demonstrated that aspirin reduced septic shock in experimental animals given infusions of endotoxin [161]. More recent investigations have used reversible intravenous cyclooxygenase blockers, principally ibuprofen. The beneficial effects of ibuprofen were most impressively demonstrated in canine [162] and primate [161] models of septic shock. Concurrent use of glucocorticoids and nonsteroidal agents appeared to provide synergistic benefits in animal models of septic shock [163].

The benefits conferred by nonsteroidal antiinflammatory agents have also been demonstrated in animal models of lung injury. In a dog model Calvin and Derbin [164] showed that pretreatment with ibuprofen increased arterial oxygenation and reduced both pulmonary vascular resistance and acute lung injury induced by glass bead embolism. Sordelli et al. [165] found that treatment with piroxicam produced dose-related increases in the recruitment of polymorphonuclear leukocytes and in the cardiovascular and peribronchial infiltration of these cells into the lungs of neutropenic mice given sublethal aerosol doses of *P. aeruginosa*. Thus treatment with piroxicam maintained pulmonary defenses against infection while decreasing damage to tissues.

Current Status of Glucocorticoids in Clinical Sepsis

The use of glucocorticoids as standard therapy in severe sepsis increased throughout the 1970s and early 1980s. Not only did data from studies in animals provide substantial sup-

port for this practice, but an important clinical study by Schumer [166] in 1976 also stimulated the use of a glucocorticoid bolus early in severe sepsis. However, after reviewing these data, the U.S. Food and Drug Administration failed to find adequate evidence to permit the Upjohn Company to continue to include severe sepsis or septic shock as an indication in its package insert for Solu-Medrol (methylprednisolone sodium succinate). Therefore, Upjohn decided to initiate a large-scale multicenter trial of methylprednisolone in the early 1980s. At the same time, the Veterans Administration Cooperative Studies Program set up a similar multicenter trial.

Meanwhile, two other significant studies were reported in 1984 [167, 168]. Hoffman et al. [167] reported on the effects of large doses of glucocorticoids on patients with severe typhoid fever. On the basis of the finding by these authors that mortality fell from 55% to 15% among patients fulfilling entry criteria, glucocorticoids continue to be indicated for the treatment of typhoid fever when patients initially fail to respond to antibiotics and volume replacement as defined in Hoffman's study. However, typhoid fever is generally thought to represent a somewhat aberrant form of gram-negative sepsis because it is reasonably well tolerated clinically and because it involves substantial activation of mononuclear as well as polymorphonuclear cell systems in host defense. In addition, Hoffman's study involved relatively healthy—though impoverished and probably malnourished—patients from the Far East whose average age was 25 years. Thus these patients probably had relatively healthy organ systems.

Sprung et al. [168] evaluated the effects of high doses of glucocorticoids on patients with frank septic shock who were being treated in an intensive care unit. This study showed no difference between the mortality figures for patients treated with steroids and those for patients given placebo. However, these patients had been in shock for an average of 18 hours before steroids were administered. Although death was delayed by a modest interval in the steroid-treated group, mortality was ~70% in both the steroid- and the placebo-treated groups.

Initial results of the 19-center Upjohn trial and the 10-center Veterans Administration studies [153, 154, 169], reported in 1987, showed no differences in outcome between steroid- and placebo-treated groups. The Upjohn study [153] used very high dosages of glucocorticoids, i.e., four doses of 30 mg of methylprednisolone/kg infused at 0, 6, 12, and 18 hours. These high doses actually increased mortality in some subgroups of patients. Mortality was significantly higher among patients with baseline creatinine values of >150 μmol/L (>2.0 mg/dL) [153] among those with adult respiratory distress syndrome (as reported separately by Bone et al. [169]). Other investigators have also reported on glucocorticoid therapy for adult respiratory distress syndrome induced by sepsis. The most recent study, also published in 1987, showed neither beneficial nor deleterious effects of high doses of glucocorticoids [170].

The Veterans Administration study [154] used a dosage ~60% as high as that used in the Upjohn study, i.e., an initial prednisolone infusion of 30 mg/kg followed by hourly infusions of 5 mg/kg for 9 hours. Thus the total dose was 75 mg/kg in the Veterans Administration trial as compared to 120 mg/kg in the Upjohn study. The Veterans Administration study showed no adverse effects of glucocorticoid therapy but some delay in the resolution of secondary infections. Again, no efficacy for steroid therapy was shown; mortality for both groups of patients was ~25%. Because patients with altered mentation were not randomized in the Veterans Administration study, patients in this trial were less sick than those in the Upjohn trial. The presence of altered sensorium as sepsis evolves substantially heightens the risk of morbidity and mortality.

In evaluating results of the Veterans Administration study, we found a time lapse of 13–22 hours between the first septic symptom and the initial infusion of glucocorticoids (Sheagren and Peduzzi, unpublished data). Thus it seems clear that clinicians simply cannot recognize the potentially septic state, identify the high-risk patients, and administer glucocorticoids in time to prevent the adverse effects of severe sepsis. A trial involving only one or two entry criteria (for example, fever and chill, fever and dyspnea, fever and tachycardia) might show that a single prednisolone dose of 30 mg/kg might speed up stabilization of the condition of patients ultimately found to be septic without adversely affecting patients ultimately found not to be septic. The prospects for such a study are obviously unlikely. In any event, the value of glucocorticoids is probably minimal in comparison to the benefits of rapid recognition, volume replacement, and antibiotic therapy.

Approaches to Treatment of the Septic Patient

Given the therapeutic modalities available to support the patient with septic syndrome, how should the clinician proceed? The following approaches are suggested.

Initial evaluation. Patients presenting with a syndrome that could be infectious must be rapidly and thoroughly evaluated. Such patients usually present with fever (with or without chills), dyspnea, tachycardia, and altered mentation, with or without decreased blood pressure and/or orthostatic changes. Some patients present without classic signs and symptoms of sepsis; in the most problematic cases, individuals remain afebrile or develop hypothermia. Sepsis is the most common cause of hypothermia in hospitalized patients [171].

History and examination. The clinician must quickly review the patient's history, looking especially for underlying immune defects (which may be predictive of infection by certain pathogens) and for microbiologic data indicating previous colonizing or infecting organisms. Known colonizing organisms in patients who develop septic syndrome must be covered by the initially administered antibiotic regimen [172].

A quick but thorough physical examination should follow review of the patient's history. Especially important is a careful inspection of intravenous line sites, skin and mucous mem-

branes, and all body orifices. Although pelvic and rectal examinations are often omitted in the cases of very ill patients, the pelvic or perineal region may be the focus of infection.

Laboratory tests. A complete white blood cell count with a differential blood cell count is mandatory. More and more laboratories are omitting differential blood cell counts unless the white blood cell count is "abnormal." Not knowing the differential blood cell count on a "normal" count may cause the clinician to miss a left shift, which occurs in sepsis even before the white blood cell count becomes elevated. Indeed, a subset of septic patients have a decreased white blood cell count. A platelet count, in which low values are highly predictive of relatively severe sepsis [173], should also be obtained. Urinalysis should be performed, and levels of blood urea nitrogen, serum creatinine, blood sugar, and serum electrolytes should be determined. The need for other blood studies is indicated by the patient's history and physical examination. Three sets of blood cultures should be performed, each set including an aerobic and an anaerobic bottle. Obviously, any peripheral sites that are likely sources of sepsis should be sampled and the materials obtained gram-stained. The gram stain continues to be the most helpful single test in guiding empiric antibiotic therapy. Special studies such as roentgenography, computed tomography, magnetic resonance imaging, echocardiography, and sonocardiography may be helpful in some situations.

Empiric antibiotic therapy. Treatment with antibiotics must be started promptly after specimens are obtained for culture. When reacting to a septic event, the clinician should attempt to have antibiotics "running" in the patient within 30 minutes after recognition of the first sign or symptom. Approaches to the use of antibiotics in severely septic patients are reviewed in the last section of this report.

Other therapeutic modalities. In the absence of effective antiinflammatory therapy, the major supportive modalities in addition to antibiotics are adequate volume infusion and debridement and drainage of septic foci. Debridement and drainage are particularly important because continued activation of inflammatory systems and other mediators contributing to the hemodynamic and organ system dysfunction of severe sepsis will occur even in the presence of antibiotics if necrotic, infected tissue remains. Once the patient's condition is stabilized, operative debridement and catheter drainage of septic foci (guided by computed tomography, ultrasonography, or other techniques) may both be mandatory. In severely septic patients the use of antiinflammatory agents or antimicrobial antibodies may also be initiated. As noted earlier, these modalities are currently being evaluated.

Future Directions in the Management f Sepsis

The septic patient will continue to challenge clinicians, and additional therapeutic modalities are being sought for use in the management of patients whose conditions cannot be stabilized by antibiotic therapy, volume administration, debridement, and drainage. Adequate maintenance of volume and perfusion will reduce the contribution of hypoperfusion to severe sepsis and multiple organ system failure. Prompt recognition of signs and symptoms remains the key to successful management of the septic patient. In the end, however, the avoidance of sepsis is, of course, far better than even the most effective diagnosis and therapy.

Antimicrobial Therapy for Gram-Negative Septicemia

Lowell S. Young

Although considerable progress has been made in the development of new antibacterial therapies, no novel compounds have been introduced for the past two decades. However, sustained emphasis on the modification and improvement of existing structures has resulted in agents of augmented potency and reduced toxicity. Most efforts have focused on the β-lactam group of agents: penicillins, cephalosporins, monobactams, carbapenems, and miscellaneous compounds including β-lactam agents paired with a β-lactamase inhibitor [174–176]. Even the quinolones that have appeared in recent years are constructed on the foundation of naphthyridine chemistry developed more than two decades ago [177].

Stratification of Patients in Clinical Trials

Because sepsis is often a complication of compromised host immunity, the stratification of patients by severity of underlying disease is critical to the interpretation of reported results of therapy [178–180]. For instance, patients with neutropenia and/or hematologic malignancy have a poorer prognosis than patients without a "rapidly fatal" underlying disease. Some earlier studies of gram-negative bacterial infection summarized the entire experience of a hospital or institution during a defined review period; thus almost all cases of gram-negative infection were included, and mortality figures represented a wide, unstratified population. Unlike these earlier studies, several recent clinical trials of new antimicrobial agents have randomized large numbers of bacteremic patients into different treatment arms, with prospective analysis of specific agents or regimens.

Evolution of Empiric Treatment

The most widely accepted categories for the stratification of host factors were proposed by McCabe and Jackson [178]. Before 1970, several major studies used the criteria set forth by those authors to stratify for underlying disease; the investigators reported mortality of 84% with "appropriate" therapy and 85% with "inappropriate" therapy [178–180]. Appropriateness was defined in terms of the susceptibility of the infecting gram-negative bacillus to one or more of the anti-

microbial agents used. Clearly, the use of therapy defined as appropriate or inappropriate by this criterion made no difference in terms of mortality.

The high mortality in these studies may not have resulted from the use of antimicrobial agents that were relatively ineffective by modern standards; instead, the low survival rates may reflect the early philosophy of initiating treatment only when blood cultures were positive. A major change in the philosophy of case management, particularly in the presence of neoplastic diseases, has since evolved. This change came about with the recognition that in overwhelming gram-negative bacteremia, such as that induced by *P. aeruginosa*, death occurs within a few days of the time when the first positive blood sample is drawn for culture [181, 182]. Thus the lack of time to alter or modify therapy led to the initiation of empiric treatment, which is now widely accepted in the management of critically ill patients.

Combination vs. Single-Drug Therapy for Gram-Negative Sepsis

For most of the 1970s, clinical evaluation of antimicrobial agents for gram-negative sepsis focused on broad-spectrum penicillins with antipseudomonal activity and combinations of these agents with aminoglycosides. Carbenicillin was the first antipseudomonal penicillin to inhibit *P. aeruginosa* in vitro, and gentamicin was the first aminoglycoside with antipseudomonal activity. However, the antipseudomonal activity of both compounds was relatively weak, and the levels of these agents in blood were only one to two times those required for the inhibition of most pseudomonal organisms. An obvious approach was to combine drugs that had relatively marginal antipseudomonal activity in anticipation of an additive or synergistic interaction [183]. Numerous studies have suggested the benefits of such combinations [184]. As we and other researchers have emphasized, careful monitoring is essential during combination therapy involving aminoglycosides. Assurance that patients are actually receiving adequate doses of the prescribed drugs is obviously crucial, but measurement of aminoglycoside levels in blood was not widespread in early clinical trials with these agents [185].

By the end of the 1970s, therapies combining antipseudomonal penicillins and aminoglycosides were resulting in overall clinical response rates of 70%–80% among patients with bacteremia [184, 186]. In prospective studies with similar protocols, we and other investigators found response rates of ≥80% when the gram-negative bacterium isolated from blood was susceptible to both of the antibiotics being used in a given combination [185]. When only one of the two drugs was active against the isolate in vitro, the response rate was 59%. When neither agent was effective in vitro, the response rate was only 20%. In studies of several agents, we and other authors have demonstrated that in vitro synergism correlates with superior therapeutic efficacy [187, 188].

The importance of host factors and underlying disease in the outcome of sepsis—and of other infectious complications—should not be overemphasized. Many prospective studies of gram-negative bacteremia have focused on neutropenic patients because this intensely managed group is usually in an excellent administrative setting for the initiation and coordination of clinical trials. Moreover, other supportive care and pharmacologic intervention tend to be relatively uniform and consistent in this setting. Among neutropenic patients the crucial variable in the outcome of sepsis appears to be recovery of the neutrophil count. Arguments relating to the efficacy of inadequacy of single-drug vs. combination therapy therefore rely heavily on the demonstrated recovery of the host's circulating neutrophil count.

The International Antimicrobial Therapy Project Group of the European Organization for Research on Treatment of Cancer has carried out several trials of empiric antibiotic therapy in febrile neutropenic patients. In an early study the group found that combinations of aminoglycosides plus penicillin were superior for the treatment of infections such as those caused by *P. aeruginosa* [189]. In its third major trial, the group found that the combination of cefotaxime with an aminoglycoside was inferior to the combination of an antipseudomonal penicillin with an aminoglycoside for the treatment of gram-negative septicemia [190]. These investigators had also observed previously that a three-drug combination of cefazolin, carbenicillin, and amikacin was inferior to a two-drug regimen from which the cephalosporin was omitted [191].

The most recent study by the European group highlighted one current controversy in empiric therapy for sepsis in neutropenic patients [192]. This trial involved ceftazidime, which probably has greater antipseudomonal activity than any other third-generation cephalosporin. In the largest-scale trial of empiric therapy in neutropenic patients, which included the largest number of bacteremic patients ever enrolled in a single study protocol, the combination of ceftazidime with a long course of amikacin (minimum, 9 days) proved superior to the combination of ceftazidime with a short course of amikacin (72 hours). The other component of this three-arm study was the combination of azlocillin plus amikacin, which proved inferior to the other two regimens.

In contrast, Pizzo and colleagues [193] at the National Institutes of Health found that initial therapy with ceftazidime alone was as effective as a three-drug regimen in a trial involving febrile neutropenic patients. However, the analysis of results included a controversial category of patients representing treatment "successes with modification." Because many such modifications were necessary in this study, the role of the initial antimicrobial therapy tended to be obscured. Moreover, relatively few patients had documented gram-negative bacteremia, and the role of recovery of the neutrophil count was not delineated.

Controversies relating to the interpretation of studies by these two groups have been highlighted elsewhere [194, 195]. Similar protocols will probably be used for future trials comparing new broad-spectrum agents with conventional thera-

pies. For now, the recent trial by the European Organization for Research on Treatment of Cancer [192], which established the efficacy of ceftazidime plus a long course of amikacin, should be considered the reference standard.

Since the introduction of ceftazidime, other agents—aztreonam, imipenem, and intravenous quinolones—have become available. The combination of two β-lactam agents may have some appeal, particularly when patients are at high risk of renal toxicity [196]. Aztreonam is particularly appealing because its structure and gram-negative spectrum resemble those of ceftazidime and because it can be used successfully for patients allergic to penicillin without the risk of severe hypersensitivity reactions [197].

Can the issue of combination vs. single-drug therapy for gram-negative sepsis be readily resolved? In several studies the use of a single-drug regimen has been successful, particularly for the treatment of seriously—but not profoundly—neutropenic patients. Patients with circulating neutrophil counts of >500/mm³ and without *P. aeruginosa* infection are likely to do well with single-drug therapy. If the agent initially chosen is effective in vitro, single-drug therapy may also be adequate for transiently neutropenic patients who are not intubated on ventilators and are not exhibiting other risk factors for gram-negative sepsis. The predictability of success will probably depend on local institutional factors, i.e., the prevalence of drug resistance. However, after several years of widespread use of some third-generation cephalosporins, evidence of resistance to newer β-lactam agents is beginning to appear [198]. This finding should serve as a caution against excessive reliance on a single agent to cover all disease-causing gram-negative bacteria.

The benefits of combination vs. single-drug therapy were evaluated in one of the most important recent studies of antimicrobial therapy in neutropenic patients. In this study De Jongh and colleagues [199] reviewed outcomes of cases of gram-negative bacteremia following the use of one or two appropriate agents and the recovery of the neutrophil count. In the presence of profound, persistent neutropenia (defined as counts of <100/μL), only patients receiving two appropriate drugs responded. If circulating neutrophil counts rose to >100/μL, however, responses were equally good whether patients received one or two effective agents. Clearly, increases in neutrophil count are unpredictable. Thus initial empiric treatment with two agents seems reasonable, with the possible discontinuation of the more toxic agent after the results of susceptibility tests become available and the neutrophil count rises. Other issues related to the use of combination chemotherapy for gram-negative bacteremia include the possibility that combinations may limit the emergence of resistance [200].

Future Directions in Antimicrobial Therapy

The likelihood that any single agent will replace drug combinations for therapy seems doubtful. Although we now have improved agents for the treatment of gram-negative infections, we also face a significant resurgence in infections due to gram-positive organisms. Thus empiric therapy must be aimed at both groups of microbes. Regimens awaiting evaluation include combinations of glycopeptides with agents that have potent activity against gram-negative bacteria as well as combinations of penicillins with intravenous quinolones.

Finally, we must ask whether the development of new agents for the treatment of gram-negative sepsis should be limited to traditional antimicrobial drugs. Elsewhere in this report, other authors have discussed the development of neutralizing antibodies and other pharmacologic interventions. Also promising is the development of cytokines, compounds that neutralize cytokines, or cytokine receptor antagonists; efforts in these directions may lead to a proliferation of useful products in the years ahead. Some of these products may stimulate regeneration of the host's bone marrow or neutralize deleterious activities of cachectin or other important mediators of gram-negative sepsis.

Clinical interest has passed from granulocyte transfusions [201] to the use of hematopoietic colony-stimulating factors [202]. Now being subjected to extensive clinical evaluation, colony-stimulating factors have the well-documented ability to stimulate bone marrow recovery [203]. Because mortality from gram-negative bacteremia is associated with persistent neutropenia, methods of decreasing the duration of neutropenia may prove important in the outcome of sepsis. Studies now under way may show that this newest area of intervention has a definite impact on morbidity and mortality. Colony-stimulating factors may also lead to intensified treatment of neoplasms and other underlying diseases involving bone marrow. Overall optimism about the potential clinical benefits of colony-stimulating factors is considerable, but the need for controlled clinical trials is obvious.

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